Effects of Mating Disruption Treatments on Navel Orangeworm (Lepidoptera: Pyralidae) Sexual Communication and Damage in Almonds and Pistachios

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ABSTRACT Two experiments in 2003 examined the effects of different ways of dispensing the principal sex pheromone component on sexual communication among and crop damage by the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) in Nonpareil almonds and pistachios. A third experiment in 2004 compared the effect on navel orangeworm damage to several almond varieties using one of these dispensing systems by itself or with phosmet, phosmet alone, and an untreated control. Additional data are presented estimating release rates from timed aerosol release devices (PuffersNOW, Suterra LLC, Bend, OR) and hand-applied membrane dispensers. In 2003, puffers placed peripherally around 16-ha blocks, evenly spaced Puffers, and hand-applied dispensers reduced males captured in virgin-baited traps by ≥95% and mating in sentinel females by ≥69%, with evenly placed Puffers showing greater reduction of males captured and females mated compared with the other dispensing systems. Mating disruption with gridded Puffers or hand-applied devices in almonds resulted in an ≈37% reduction of navel orangeworm damage (not significant), whereas peripheral Puffers resulted in a 16% reduction of navel orangeworm damage to almonds. In pistachios neither peripheral nor gridded Puffers reduced navel orangeworm damage, whereas insecticide reduced damage by 56%. In 2004, Puffers alone, insecticide alone, and both in combination significantly reduced navel orangeworm damage in Nonpareil almonds. In other, later harvested varieties, the insecticide treatments reduced damage, whereas the mating disruption treatment alone did not. We discuss application of these findings to management of navel orangeworm in these two crops.

KEY WORDS mating disruption, almonds, pistachios, *Amyelois transitella*

The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), is a key pest of both almonds, *Prunis amygdalus* Batsch, and pistachios, *Pistacia vera* L., in California (Wade 1961, Bentley et al. 2003, Zalom et al. 2005). Current practices for control of navel orangeworm damage in almonds and pistachios are intimately linked to cultural practices in these crops. Almonds are usually grown with different varieties in alternating rows. Often the variety Nonpareil comprises 50% of the orchard, and the other 50% is one or more of several varieties collectively referred to as pollenizers. Nonpareil almonds typically mature first and are harvested sometime in August, several weeks ahead of the pollenizer varieties. Approximately 40–60 d before harvest, the hulls begin to split, exposing the nut inside the shell. The risk of navel orangeworm infestation in almonds is very low before hullsplit, and increases markedly thereafter. The shell of Nonpareil almonds is relatively light and porous, and the nut meat is considered more susceptible to infestation compared with "hard shell" varieties (Soderstrom 1977). Pistachios have a single commercially important variety, Kerman, and splitting of the hull occurs much closer to harvest, if at all. Harvest of both crops results in a significant number of nuts remaining to serve as host material for development and overwintering of navel orangeworm.

The development of mating disruption for suppression of navel orangeworm damage has progressed slowly. Until recently, only the principal component of the female sex pheromone was known (Coffelt et al. 1979, Leal et al. 2005). This component, (Z,Z)-11,13-hexadecadianal (Z11,Z13-16:Ald), is not sufficient to efficiently bring males to a point source (Leal et al. 2005) and is particularly vulnerable to degradation in the field (Curtis et al. 1985).

Mating disruption formulations in current commercial use have been broadly categorized as microencapsulated dispensers, hand-applied dispensers, and high-emission dispensers (Sarfraz et al. 2006). Previously trials with Z11,Z13-16:Ald released from hand-applied dispenser in 6–8-ha plots demonstrated

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greatly reduced capture of males in virgin-baited flight traps and reduction of mating in sentinel females (Curtis et al. 1985). In two of four trials, significant reduction of crop damage resulted. The dispensers were replaced 3–5 times over ≈85 d, and a total of ≈12.5 g of Z11,Z13-16:Ald per ha was placed in the field (theoretically 146 mg/ha/d), but most of this material was never released (Curtis et al. 1985). A high-emission dispenser, in which the pheromone is stored in a liquid organic solvent before being released at timed intervals, reduced this problem (Shorey and Gerber 1996). Studies with this and other lepidopteran pests have suggested that these high-emission dispensers (Puffers) placed around the perimeter of plots of up to 16 ha can reduce males captured in pheromone traps as effectively as such devices placed evenly throughout the plot, thereby saving labor costs (Shorey and Gerber 1996, Shorey et al. 1996).

Mating disruption treatment blocks of ≥16 ha were previously recommended (Shorey and Gerber 1996) because the navel orangeworm has a high capacity for dispersal. A mark–release–recapture study examining eggs colored by fat soluble dye in the maternal diet found that, over two to four nights after eclosion, females oviposited equally at all distances up to 375 m from the release point (i.e., an area of 44 ha) (Andrews et al. 1980). However, sanitation trials indicated that 20-ha almond blocks were large enough to obtain benefit from sanitation despite being surrounded by unsanitized almonds (Curtis 1976), suggesting that blocks around this size were large enough to avoid obscuring the effect of treatments within the block by immigration from outside the block.

Here, we present results of experiments in 2003 and 2004 comparing the effects of release systems for Z11,Z13-16:Ald on sexual communication and nut damage in almonds and pistachios located across the southern San Joaquin Valley. The objective of this study was to 1) to compare changes in release rate over time, under field conditions, between a hand-applied membrane dispenser (CheckmateNOW, Suterra LLC, Bend, OR) and Puffers (PufferNOW, Suterra LLC, Bend, OR); 2) to compare effect on sexual communication and damage of peripherally-placed Puffers with Puffers or hand-applied devices placed evenly throughout the plot; and 3) to further examine evenly spaced Puffers alone or in combination with insecticide on navel orangeworm damage in several almond varieties.

Materials and Methods

General Procedures. Traps using virgin females as a pheromone source were used to monitor relative abundance of males and their ability to locate females. Groups of three females were sealed in a mesh bag, which was then suspended from inside of a wing trap (Pherocon IC, Trécé Inc., Adair, OK). Females placed in the field in this manner were shown previously to live and call for 4–7 d in summer conditions in this region (Burks and Brandl 2004). Moths for this experiment were obtained as eggs from a laboratory colony originally obtained in 1966 from the University of California, Berkeley, and maintained on a wheat bran diet (Tebbets et al. 1978). Three newly eclosed females were enclosed in plastic mesh bags and placed in traps in the field within 24 h. Traps were checked and females replaced weekly, and liners were removed and replaced if they contained moths or were dirty. Old liners were taken to the laboratory to confirm field counts and identification.

Effects of the treatments on mating were examined with sentinel females using methods and apparatus similar to those described by Curtis et al. 1984). A 473-ml round polypropylene cup was suspended from the top of a wing trap by clips, and used to contain a second such cup with the top half coated with Fluon (ICI, London, United Kingdom). A portion of the wings on one side were clipped on freshly eclosed females, which were individually placed in plastic vials for transport the same day to mating assay locations in the field. The next week, females were again placed in plastic vials for transport to the laboratory where they were evaluated for mating based on the presence or absence of spermatophore(s) in the bursa.

The ranches used were owned and managed by Paramount Farming (Bakersfield, CA), and their location codes are used here to distinguish these sites. Four of these ranches (3410, 3710, 3740, and 3940) contained almonds of the varieties Nonpareil, Carmel, Fritz, and Monterey, and four (4010, 4260, 4540, and 4840) contained Kerman pistachios. These ranches were within a 65 by 31 km (east–west and north–south, respectively) area of Kern County, CA; an area roughly encompassed by Highways 99 and 33 on the east and west, respectively, and Seventh Standard Road and County Line Road on the south and north, respectively. The distance between each of these ranches and the next nearest location in this study ranged from 3 to 14 km, with a median of 5 km.

Percentage of reduction of males captured in virgin female-baited traps was calculated as [1 − (no. males captured in pheromone plot/no. males captured in untreated) × 100]. SAS software (SAS Institute 2004) was used for all analysis, and proportions of nuts with harvest damage were transformed as $\arcsin(\sqrt{x})$ before analysis.

Release Rate of Hand-Applied Dispensers and Puffers under Field Conditions. Concurrently with the two mating disruption experiments in 2003 examining effects of mating disruption treatments on sexual communication (see below), an experiment was performed examining rate of loss of Z11,Z13-16:Ald from hand-applied dispensers under field conditions. Dispensers were placed on the north and south side of almond trees far from mating disruption experiments, and removed from 10 trees at various intervals for analysis. One set of dispensers was placed in the field on 26 March and sampled 20, 41, 54, 68, 96, 110, 155, 202, and 225 d later, and a second set was placed in the field on 17 July and sampled on days 21, 42, 56, 89, and 112. Gas chromatography was used to determine the amount of Z11,Z13-16:Ald that could be extracted from the dispensers. The difference between phero-

mone content of hand-applied dispensers removed from the north and south side of trees on the same day was compared using Student's t test (TTEST procedure), with a Bonferroni correction for multiple comparisons (Zar 1999). The average rate of emission over each of the intervals was estimated as the difference in average Z11,Z13-16:Ald content divided by the interval in days, emission rate versus time (d) fit to first-order decay {mg Z11,Z13-16:Ald = $a \times \exp(-b*d)$} and linear equations {mg Z11,Z13 = $a - b*d$} by using the NLIN and REG procedures. Changes in Z11,Z13-16:Ald content also were compared over the first 115 d between the first and second application by using linear and first-order decay equations, and the predicted mean and 95% confidence limits (CL) for the decay equation were used to estimate the hourly rate of pheromone release over the season.

In 2003, the emission rate from timed release aerosol dispensers (Puffers) was examined by determining the crude weight of a single puff 77 d after activation in two almond blocks ($n = 79$ and 59 Puffers), and 99 d after activation in an almond and a pistachio block ($n = 155$ and 161, respectively). The crude weight was multiplied by the proportion and purity of Z11,Z13-16:Ald in the canister. Fixed effects analysis of variance (ANOVA) (GLM) with a Tukey–Kramer adjustment for multiple comparisons was used to examine the differences between the four block-time combinations.

In 2004, procedures similar to those for hand applied dispensers in 2003 were used to examine changes in emission rate of Puffers over the season. The Puffer consisted of a cabinet containing a programmable electronic timer and an aerosol canister. Aerosol canisters used in 328 Puffers as part of an experiment (see below) were weighed when first placed in service, weighed again after 30–50 d, and then weighed after four to five additional intervals of 27–30 d. The difference in weight, divided by the interval and multiplied by the proportion Z11,Z13-16:Ald, was used as an estimate of the emission rate over this interval. Fixed effects ANOVA with Dunnett's test for difference of means from a control was used to examine differences in emission rate over time, with the average daily pheromone emission as the dependent variable and time (in days) between Puffer activation and the interval midpoint as the independent variable.

To facilitate comparison between the emission rate of Puffers and membrane dispensers, estimates of the mean and standard error of the hourly release rate per dispenser in both 2003 and 2004 were multiplied by the density of dispensers per hectare and reported as milligrams per hour per hectare.

Comparison of Mating Disruption Treatments in Almonds and Pistachios in 2003. Two similar randomized complete block mating disruption experiments were performed in almonds and pistachios. Four treatments were applied to 16-ha treatment plots centered in each corner of four blocks, making up square 256-ha plantings of almonds or pistachios (i.e., a part or all of a ranch) (Fig. 1). In the first mating disruption experiment, in almonds, treatments included 1) a con-
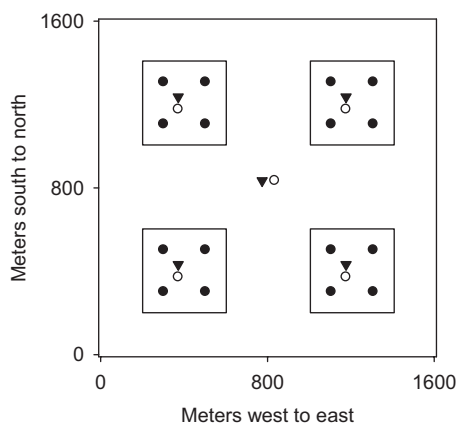

Fig. 1. Plot arrangement for randomized complete block design in almonds and pistachios in 2003, representing one of four 256-ha blocks (replicates) each in almonds and pistachios. The smaller squares represent 16-ha treatment plots. Effects of treatments were compared using wing traps baited with virgin females (dark circles), unbaited wing traps (dark triangles), and sentinel females (open circles). A blank wing trap and sentinel female were placed in the center of the block between the treatment plots.

trol that received no residual insecticide or mating disruption treatment targeted against navel orangeworm, 2) mating disruption with Puffers placed around the perimeter of the treatment plot, 3) mating disruption with Puffers placed in an even grid throughout the block, and 4) mating disruption with hand-applied membrane dispensers placed on each tree in the block. A 96-ha limit on experimental treatments precluded us from using hand-applied dispensers in both almonds and pistachios, so in the second mating disruption experiment, in pistachios, one of the four treatment blocks was instead treated 30 d before harvest with a residual insecticide, azinphosmethyl (Guthion 50 WP, Research Triangle Park, NC) at a rate of 2.26 kg of active ingredient (AI) in 1,893 liters water per ha. Response variables for each of the two experiments were males captured in traps baited with virgin females, mating status in sentinel females, and navel orangeworm damage to harvest samples of Nonpareil almonds.

Mating disruption treatments were applied from 3 April through mid-October in almonds or mid-September in pistachios. Puffers were placed peripherally or evenly throughout the experimental block at a density of five dispensers per ha, and emitted a target of 40 mg of solvent and propellant containing 1.09% Z11,Z13-16:Ald of 87.4% purity, for a target of 0.38 mg of Z11,Z13-16:Ald every 15 min from 6 PM to 6 AM (PDT) (i.e., 91 mg/ha/d Z11,Z13-16:Ald over 160–200 d). Hand-applied dispensers were placed in the trees at a rate of 375 per ha (two per tree) on 26 March, a second and equal application was placed on 17 July.

Disruption of male trap capture was compared between treatments by using four virgin-baited wing traps in each 16-ha treatment plot, placed 1.5 m above the ground, 200 m from the nearest other traps in the

plot, and 100 m from the edge of the block (Fig. 1). The distance of these traps from the nearest Puffer in any direction was ≥24 m in the gridded Puffers and ≥95 m for the peripherally placed Puffers. Blank flight traps, without virgin females, also were placed at the center of each treatment plot and at the center of the 256 ha ranch (block). These data were collected for 24 consecutive weeks in pistachios and 23 wk in almonds, from 31 March to 8 September 2003. We used sentinel females, placed at the center of each treatment plot and in the center of the ranch, to compare the relative probability of female mating.

Impact of the treatments on crop damage was compared by taking nut samples at 16 points within each of the plots, which were pooled to form samples of 4,265–7,967 almonds and 10,738–19,310 pistachios per plot. Each nut was opened and examined under magnification by Paramount Farming research personnel. Harvest dates for Nonpareil almonds were 17 and 21 August for ranches 3940 and 3740, and 28 August for ranches 3440 and 3710. Harvest dates for pistachios were 16 and 17 September for ranches 4010 and 4510 and 24 and 25 September for ranches 4260 and 4840.

The effect of treatments on males captured in virgin female baited traps in almonds and pistachios over the 23–24-wk observation period was analyzed using generalized linear mixed models (GLIMMIX) with a negative binomial distribution (Agresti 2007). The dependent variable was the sum of males captured in plots in almonds and pistachios over the 23–24-wk observation period, ranch was a random effect, the mating disruption or other treatment was a fixed effect, and a Tukey adjustment was used for multiple comparisons. The number of males captured in blank traps was negligible in both almonds and pistachios, and blank traps were therefore not included in the analysis. Differences in female mating were examined using contingency table analysis for multiple proportions (FREQ) with a Tukey style test for multiple comparisons (Zar 1999). Navel orangeworm damage was compared using a 2-way mixed model ANOVA (MIXED) in almonds and pistachios, with treatment as a fixed effect and ranch as a random effect.

Comparison of Mating Disruption in Almonds with and without Insecticide Treatments in 2004. A third mating disruption experiment in 2004 examined the effect of mating disruption with gridded Puffers in several almond varieties, alone or with a hullsplit insecticide treatment. Treatments were 1) untreated controls, 2) mating disruption with gridded Puffers, 3) a hullsplit treatment with residual insecticides, and 4) a combination of both the mating disruption and residual insecticide treatments. Response variables for this experiment were males captured in traps baited with virgin females, mating status in sentinel females, and navel orangeworm damage to harvest samples. Treatment effects on navel orangeworm damage were examined for Nonpareil and two pollenizer varieties, Carmel and Monterey.

A Latin square experimental design (Zar 1999) was used in Ranch 3710 to obtain greater replication within an area of homogenously high navel orange-
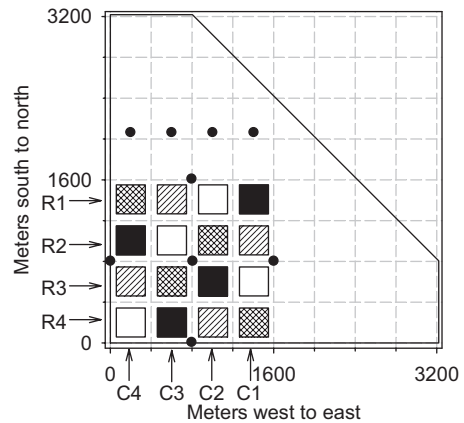

Fig. 2. Plot arrangement for Latin square experiment in almonds in 2004. Squares represent 8-ha treatment plots, with untreated control plots represented by white squares, plots treated with Puffers represented by diagonal lines, plots treated with insecticide represented by cross hatches, and plots treated with both mating disruption and insecticide represented by black squares. Row and columns grouping of plots (Table 4) are indicated by the letters and arrows. Wing traps baited with virgin females outside of the Latin square test area and within the area outside the plots are represented by dark circles. There were four virgin-baited flight traps in each treatment plot, arranged as in Fig. 1.

worm activity. A square 256-ha block was divided into 16 square eight ha (284 by 284 m) plots separated from each other by 118 m, and from the sides of the block by 59 m (Fig. 2). Each treatment was represented once in each east–west row and once in each north–south column. The 256-ha block of almonds examined was bordered by additional sections of almonds to the north and east, and by highways to the south and west. Puffers were placed at two-thirds canopy height evenly throughout the treated plots at a density of five per ha, and mating disruption with 105 mg/ha/d Z11,Z13-16:Ald was applied from the beginning of March until the end of the Monterey harvest. A hullsplit insecticide treatment consisting of 4.18 kg of phosmet (AI) (Imidan 70 WP, Gowan, Yuma, AZ) and 113 g of permethrin (AI) (Pounce 3.2 EC, FMC Agricultural Products, Philadelphia, PA) in 1,893 liters water per ha, was applied on 10 July. Areas between the treatment plots simultaneously received a hullsplit insecticide treatment with 4.18 kg of phosmet (AI) in 1,893 liters water per ha but without permethrin.

Traps baited with virgin females were used to compare disruption of male trap captures inside treatment plots, and in the buffer regions between treatment plots, with activity in an adjacent block of almonds completely outside the 4 by 4 grid of treatment plots (Fig. 2). Within each treatment, plot four wing traps were placed 142 m from each other and 71 m from the side of the treatment block. In mating disruption treatment blocks, these traps were ≥15 m from the nearest Puffer and ≥18 m from the nearest upwind Puffer. Outside the 256-ha site containing the 16 treatment plots, four traps were placed 500 m north of the north

edge and parallel with the center of each of the four columns of treatment plots (Fig. 2). Within this site, a trap was placed in the center of the section and one each in the middle of the west, north, east and south sides of the section 46 m from the edge. Females were placed in the flight traps on 1 March, 2004 and replaced weekly until 24 August.

Samples of Nonpareil almonds were taken from the ground after trees were shaken for harvest and dislodged nuts had been piled in the center of alleys for harvest. Dates of harvest were 20–23 August for Nonpareil, 17 September for Carmel, and 27 September to 4 August for Monterey. Samples of 474 ± 6.4 (mean $\pm$ SD) Nonpareil, 89 ± 1.2 Carmel, and 86 ± 1.2 Monterey almonds each were taken from a point in a windrow within 16 equal sectors in each treatment plot. These 16 subsamples for each variety were pooled for determination of infestation as described previously.

The GLIMMIX procedure was used to perform generalized linear models analysis, with a negative binomial distribution and all fixed effects, for comparison of treatment effects on males captured in virgin female-baited traps. The dependent variable was total males captured in the four traps in each plot over the monitoring period, and the independent variables were row, column, and treatment. Fixed effects ANOVA was used to examine row, column and treatment effects on navel orangeworm damage to almonds in the treatment plots at harvest, and Fisher protected least significant difference (LSD) was used for separation of means. Separate analyses were performed for damage in Nonpareil, Carmel, and Monterey almonds.

Results

Release Rate of Hand-Applied Dispensers under Field Conditions. The hand-applied dispensers released a calculated 32.3 g/ha Z11,Z13-16:Ald over the season. Nonlinear regression of estimates of emission rate on time in field by using a first-order decay model was significant ($F = 40.19$; df = 2, 24; $P < 0.0001$) and accounted for much of the observed variation ($r^2 = 0.77$). Estimates of the initial rate and the decay were 0.603 ± 0.12 mg/d (mean $\pm$ SE) and -0.0096 ± 0.0035 mg/d/d, respectively. Dispensers on the south side of the tree lost significantly more Z11,Z13-16:Ald ($P < 0.05$ after Bonferroni adjustment) than those on the north side on days 54, 68, and 96 of the first application (19 May to 30 June), but not at any time in the second application (Fig. 3A). The first-order decay equation fit the data for changes in membrane weight over the first 115 d better than linear regression ($r^2 = 0.99$ versus 0.89–0.91), and parameter estimates were not significantly different between the two applications with either linear or nonlinear regression. The daily mean release rate, as estimated using first order decay, is plotted in Fig. 3A for the first and second applications of hand-applied devices.

In 2003, the single-puff emission rate for Puffers in the almond block was 8.5 ± 0.17 and 8.8 ± 0.23 mg/ha/h Z11,Z13-16:Ald in the two almond blocks on day
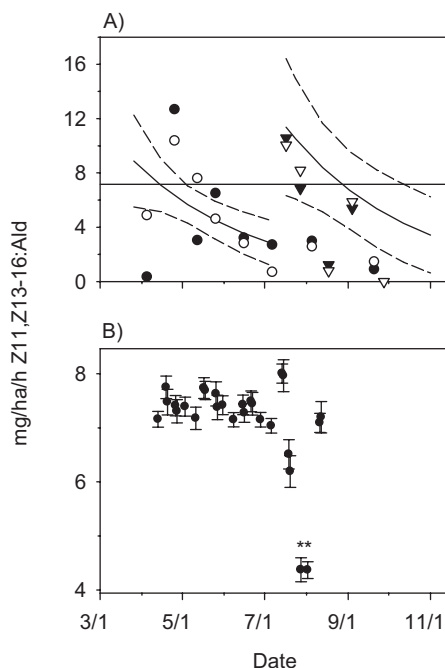

Fig. 3. Effect of time in field on the emission rate of two types of dispensers. (A) Hand-applied dispensers in 2003: Estimated emission rate of groups of 10 membranes placed in the field on 26 March (circles) or 17 July (triangles) on the north (white symbols) or south (black symbols) side of the tree. Solid and dashed lines indicate the mean and 95% CI predicted by nonlinear regression for dispensers from the first application before 17 July, and from combined emission from dispensers from both applications after 17 July. The vertical reference is the mean for the first rate determined for Puffers in 2004. (B) Emission of Puffers between 7 AM and 7 PM (PDT) in 2004 (mean and SE). There were no significant changes in the rate of emission for the first 120 d in the field. Observations with asterisks differ significantly ($P < 0.05$) from the first rate determined (day 35). Note that the vertical axis differs between A and B.

77 and 8.7 ± 0.14 and 7.3 ± 0.23 mg/ha/d Z11,Z13-16:Ald in almonds and pistachios, respectively, on day 99 (i.e., 88–106 mg/ha/d). There were significant differences among these means ($F = 20.34$, df = 3, 450; $P < 0.0001$). The pistachios on day 99 were significantly different than the other three measurements, which were not significantly different from each other.

In 2004, 93% of the Puffer units functioned throughout the entire season with no maintenance. The most common maintenance was replacing Puffer canisters, which occurred most frequently on days 141 and 146. There were significant differences in emission rates between days ($F = 24.79$, df = 26, 1,380; $P < 0.0001$). Emission rates were significantly less than the reference value (i.e., the first measurement) on days 141 and 146 ($P < 0.0001$) (Fig. 3B).

Comparison of Mating Disruption Treatments in Almonds and Pistachios in 2003. There were differences between mating disruption treatments in al-

Table 1. Treatment and replicate effects (mean ± SE) on navel orangeworm males captured per plot in 16-ha plots in almonds between 31 March and 15 September 2003

Factor	Level	Males per plot	% reduction
Treatment	Gridded Puffers	2.5 ± 0.9a	99.7
	Peripheral Puffers	13.5 ± 11.5ab	98.2
	Hand-applied dispensers	31.3 ± 12.6b	95.7
	Untreated	729.8 ± 383.5c	
Replicate	Ranch 3740	34 ± 18	
	Ranch 3440	118 ± 114	
	Ranch 3940	139 ± 133	
	Ranch 3710	487 ± 451	

Means followed by different letters are significantly different ($P <$ 0.05).

monds in the number of males captured in virgin-baited traps ($F = 26.10$, df = 3, 9; $P < 0.0001$) (Table 1). Significantly fewer males were captured in all mating disruption treatment plots compared with untreated controls, significantly fewer males were captured in plots treated with gridded Puffers compared with peripheral Puffers, and the number of males captured in plots treated with hand-applied dispensers was not significantly different from those captured in plots treated with either gridded or peripheral Puffers (Table 1). A plot of trap sums by week shows that, in almonds, most males in virgin-baited traps were captured after 1 August and, before 24 August, more males were captured in plots treated with peripheral Puffers compared with hand-applied dispensers (Fig. 4). Over the observation period, three males were captured in blank traps in almonds, compared with the 10 females captured in female-baited traps in gridded Puffer plots.

In pistachios, there were significant differences between treatments in sums of males captured in virgin-baited flight traps ($F = 76.21$, df = 3, 9; $P < 0.0001$). There were significantly fewer males captured in plots treated with gridded Puffers than in those treated with peripheral Puffers, and far fewer males were captured in plots treated with either of the mating disruption treatments compared with the untreated control or the pesticide treatment (Table 2). Over the observation period, six males were captured in blank traps in pistachios compared with the 44 males in female-baited traps in gridded Puffer plots.

There were significant differences between treatments in the proportion of sentinel females mated in both almonds ($\chi^2 = 33.17$, df = 4, $P < 0.0001$) and pistachios ($\chi^2 = 90.26$, df = 4, $P < 0.0001$). In almonds, significantly fewer females were mated in the plots treated with peripheral or gridded Puffers compared with females in the untreated control plot or in the center between plots, whereas the proportion of females mated in plots treated with hand-applied dispensers were not significantly different from that in either the Puffer-treated plots or the untreated control plots (Table 3). In pistachios significantly more females were mated in the untreated control plot compared with the center position and plots treated
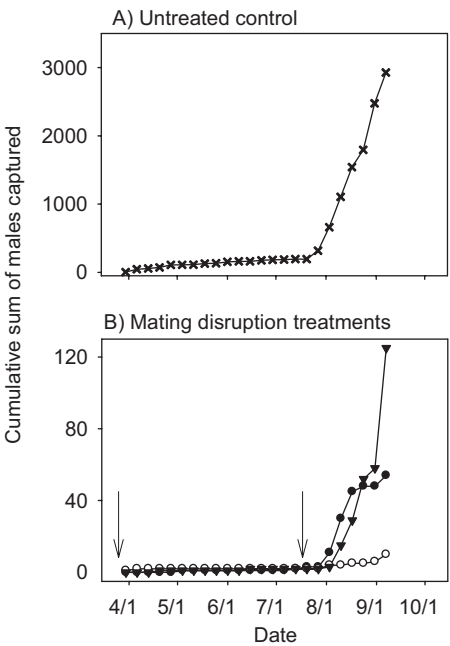

Fig. 4. Cumulative sum of navel orangeworm males captured in virgin female-baited traps in almonds in 2003 in untreated control plots (A) and mating disruption plots. (B). Symbols: males captured in untreated control plots (Xs); males captured in plots treated with peripherally-placed Puffers (black circles); males captured in plots treated with hand-applied dispensers (black triangles); and males captured in plots treated with gridded Puffers (white circles). Arrows indicate dates of first and second applications of the hand-applied dispensers.

with either gridded or peripheral Puffers had significantly fewer females were mated compared with any nonmating disruption treatment (Table 3).

The percentage of almonds damaged by navel orangeworm was 3.6 ± 2.8 (mean ± SE), 3.0 ± 2.4, 2.3 ± 1.6, and 2.3 ± 1.7% in the untreated control, peripheral Puffer, hand-applied dispenser, and gridded Puffer plots, respectively. A mixed models ANOVA found no significant differences among these treatments ($F = 1.80$, df = 3, 9; $P = 0.2178$). The mean navel orange-

Table 2. Treatment and replicate effects (mean ± SE) on navel orangeworm males captured per plot in 16-ha plots in pistachios between 31 March and 8 September 2003

Factor	Level	Males per plot	% reduction from untreated
Treatment	Gridded Puffers	11 ± 3a	99.3
	Peripheral Puffers	72 ± 35b	95.7
	Azinphosmethyl	2,853 ± 449c	
	Untreated	1,662 ± 456c	
Replicate	Ranch 4260	965 ± 766	
	Ranch 4010	1,013 ± 592	
	Ranch 4510	1,019 ± 655	
	Ranch 4840	1,601 ± 909	

Means followed by different letters are significantly different ($P <$ 0.05).

Table 3. Effect of treatments on mating status of sentinel females recovered from mating assays in 2003 in the center of each 16-ha treatment plot and in the center of the 256-ha block, equidistant between the treatment plots

Mating disruption?	Treatment	Almond		Pistachio	
		n	% mated	n	% mated
No	Center of 256-ha block	81	14.8ab	76	35.5b
	Untreated control	83	19.3a	81	61.0a
	Azinphosmethyl			77	53.1ab
Yes	Hand-applied dispensers	84	3.6bc		
	Peripheral Puffers	84	2.4c	81	11.4c
	Gridded Puffers	86	0.0c	79	3.8c

Percentages within the same column followed by different letters are significantly different ($P < 0.05$).

worm damage to almonds (across treatments) was $9.1 \pm 1.2\%$ in ranch 371 and ranged from 0.2 to 1.1% in the remaining three ranches. In pistachios there were significant differences in navel orangeworm damage between treatments ($F = 5.70$, df = 3, 9; $P = 0.0182$). The percentage of pistachios with navel orangeworm damage was 1.2 ± 0.33, 1.3 ± 0.39, 1.2 ± 0.31, and $0.5 \pm 0.13\%$ in the untreated control, peripheral Puffer, gridded Puffer, and insecticide-treated plots, respectively. There was significantly less damage in insecticide-treated pistachios ($P < 0.0001$), but no significant differences in damage among the remaining treatments. The mean percentage of navel orangeworm damage by ranch ranged from 0.4 to 1.6%.

Comparison of Mating Disruption in Almonds with and without Insecticide Treatments in 2004. Over the 26-wk monitoring period, $1,287 \pm 110$ males (mean $\pm$ SE) per trap were captured in four traps in an adjacent block of almonds, compared with 44 ± 21 males captured in five traps between treatment plots in the Latin square plot arrangement. Within the 16 plots, there were significant differences in the sum of males captured over the season between treatments ($F = 54.58$, df = 3, 6; $P < 0.0001$), but not between rows ($F = 2.38$, df = 3, 6; $P = 0.1688$) or columns ($F = 2.85$, df = 3, 6; $P = 0.1274$). There were significant difference in males

captured between mating disruption and nonmating disruption plots, but not between the untreated control and hullsplit insecticide treatment or between plots treated with mating disruption with or without a hullsplit insecticide treatment (Table 4).

For navel orangeworm damage to Nonpareil almonds at harvest, the ANOVA model was significant ($F = 10.21$, df = 9, 6; $P = 0.0052$). There were significant differences in the proportion of Nonpareil almonds with navel orangeworm damage between treatments ($F = 18.99$, df = 3, 6; $P = 0.0018$) and columns ($F = 8.12$, df = 3, 6; $P = 0.0156$), but not between rows ($F = 3.53$, df = 3, 6; $P = 0.0884$). The untreated control had significantly greater damage than all other treatments, there was no difference in Nonpareil damage between the hullsplit treatment with or without gridded Puffers, and the gridded Puffer treatment by itself had intermediate damage (Table 4).

For navel orangeworm damage to Carmel almonds at harvest the ANOVA model was significant ($F = 5.21$, df = 9, 6; $P = 0.0300$). There were significant differences in the proportion of Carmel almonds with navel orangeworm damage between treatments ($F = 9.75$, df = 3, 6; $P = 0.0101$), but not between columns ($F = 3.19$, df = 3, 6; $P = 0.1052$) or rows ($F = 2.40$, df = 3, 6; $P = 0.1666$). The plots treated with a hullsplit insecticide, with or without the gridded Puffer treatment, had less navel orangeworm damage than the untreated control, whereas there was no significant difference in damage to Carmel almonds between the gridded Puffer treatment by itself and the untreated control. The same pattern as for Carmel was seen in damage to Monterey almonds (Table 4). In that case, the overall model was significant ($F = 9.35$, df = 9, 6; $P = 0.0300$), and there were significant differences between treatments ($F = 19.60$, df = 3, 6; $P = 0.0017$), but not between columns ($F = 3.96$, df = 3, 6; $P = 0.0714$) or rows ($F = 4.50$, df = 3, 6; $P = 0.0559$).

Discussion

These data demonstrate that mating disruption can significantly reduce navel orangeworm damage in al-

Table 4. Latin square experiment in almonds in 2004: treatment, row, and column effects on males (mean $\pm$ SE) captured in virgin female baited traps and navel orangeworm damage in Nonpareil (NP), Carmel (Ca), and Monterey (Mo) almonds at harvest

Factor	Level	Males per trap	% dmg NP	% dmg Ca	% dmg Mo
Treatment	Untreated	$97.56 \pm 22.71a$	$6.46 \pm 1.19a$	$7.69 \pm 2.52a$	$10.58 \pm 1.86a$
	Phosmet + permethrin	$187.13 \pm 51.75a$	$3.00 \pm 0.17c$	$1.87 \pm 0.20b$	$4.81 \pm 0.69b$
	Puffers	$0.44 \pm 0.22b$	$4.52 \pm 1.04b$	$5.16 \pm 0.45a$	$9.52 \pm 1.69a$
	Both	$0.31 \pm 0.12b$	$2.46 \pm 0.27c$	$2.22 \pm 0.51b$	$3.79 \pm 0.63b$
Row	Row 1 (north)	145 ± 53	4.54 ± 0.39	5.77 ± 2.28	8.91 ± 2.37
	Row 2	18 ± 6	4.59 ± 0.99	3.70 ± 1.08	7.06 ± 2.00
	Row 3	38 ± 19	4.36 ± 1.38	4.99 ± 2.34	7.83 ± 2.31
	Row 4 (south)	85 ± 32	2.94 ± 0.41	2.49 ± 0.62	4.90 ± 0.85
Column	Column 1 (west)	121 ± 52	$3.12 \pm 0.39b$	2.44 ± 0.71	5.26 ± 0.79
	Column 2	13 ± 5	$5.31 \pm 1.52a$	3.50 ± 1.13	7.85 ± 2.14
	Column 3	44 ± 22	$3.02 \pm 0.63b$	5.04 ± 2.41	6.57 ± 2.25
	Column 4 (east)	107 ± 33	$4.98 \pm 1.22a$	5.96 ± 2.07	9.03 ± 2.38

Means followed by different letters in the same column and level-within-factor grouping are significantly different ($P < 0.05$). Rows and columns are as shown in Fig. 2. There were no significant differences among row means of damage to Nonpareil, and among row and column means of damage to Carmel and Monterey.

monds. The first mating disruption experiment, in almonds in 2003, found mean reductions of navel orangeworm damage to Nonpareil almonds of 16% by peripheral Puffers and 37% by either hand-applied membranes or gridded Puffers. Because of high variation due to very low damage at three of the four sites, none of these differences were statistically significant. The third mating disruption experiment, in almonds in 2004, found a 31% reduction in navel orangeworm damage to Nonpareil almonds using gridded Puffers, and this difference was statistically significant.

The second mating disruption experiment, in pistachios in 2003, did not demonstrate reduction of navel orangeworm damage in pistachios treated with the mating disruption technique that was most effective in almonds (i.e., gridded Puffers). The trap data (males per replicate and males per plot in the untreated plots) suggest that males were significantly more abundant in the pistachio sites than in the almond sites that were examined in 2003. Other data indicate that this greater abundance in pistachios compared with almonds is a general trend in the southern San Joaquin Valley (our unpublished data). In contrast to the abundance data, navel orangeworm damage to pistachios, although economically important, is generally more limited in worse-case situations compared with almonds (B.S.H., unpublished data). Previous studies have commented on the relative susceptibility of Nonpareil almonds to navel orangeworm infestation (Soderstrom 1977), and lesser susceptibility of pistachios (Crane 1978). These observations suggest that navel orangeworm infestation in almonds, particularly Nonpareil, is due to a relatively susceptible crop exposed to moderate abundance, whereas in pistachios it is due to a less susceptible crop exposed to higher abundance. Mating disruption often works better with low initial abundance (Cardé and Minks 1995); therefore, this difference in the relationship between abundance and damage in the two crops suggests that using mating disruption for reduction of navel orangeworm damage in pistachios will be a greater challenge compared with almonds. However, the nonbearing period for pistachios (Klonsky et al. 1998) is greater than that for almonds (Kester and Asay 1975), and it is possible that use of mating disruption in pistachios beginning in the early years in the life of the orchard would prevent the development of a potentially damaging navel orangeworm population.

Peripheral placement of Puffers around control blocks has been suggested as a method of reducing treatment cost (Shorey and Gerber 1996). This study, like others (Shorey and Gerber 1996, Shorey et al. 1996, Burks and Brandl 2004), found that Puffers placed peripherally around 16-ha blocks significantly reduced males captured in traps baited with virgin females, thereby demonstrating interference with sexual communication. But the male trapping data from pistachios, where these treatments were challenged with higher abundance, shows 96% reduction of trap capture with peripheral Puffers versus 99% reduction with gridded Puffers, a significant difference. In 2003, sentinel females in the center position between the plots were mated less frequently than those in the untreated control plot; the control plot was also farther from plots receiving mating disruption treatments. In 2004, the average of the mating disruption treatments with or without hullsplit insecticide (targeted against larvae) represented a 99.7% reduction in males captured versus non-MD treatments. By comparison, the traps between the treatment plots showed a 70% reduction, and the traps far outside the test area captured 9x more males. We recognize that these comparisons are complicated by unequal numbers and spacing of the between-plot and outside traps compared with those in the treatment plots. These data nonetheless suggest that male trap capture was depressed throughout the area of the 2004 Latin square experiment. The data from the Latin square experiment also suggested a pattern of fewer males captured in the inner treatment plots compared with the outer plots (i.e., rows and columns two and three versus one and 4), whereas no such pattern was evident in the damage data from these plots. These observations indicate that Puffers are able to reduce males captured in flight traps baited with virgin females and the proportion of sentinel females mated over greater distances than they are able to influence navel orangeworm damage to almonds. We conclude that the peripheral Puffer arrangement, as proposed previously (Shorey et al. 1996), should not be considered further.

Puffers and the hand-applied membrane dispensers used in this study represent very different ways of implementing mating disruption, with different advantages and disadvantages in terms of mechanism and equipment and labor costs (Sarfraz et al. 2006). The hand-applied dispensers are simple and relatively economic devices that release pheromone steadily at a continuously declining rate, as demonstrated in Fig. 3A. Logically, hand-applied devices might be more sensitive to temperature, which would explain the low emission rate estimates in April and the greater difference in emission rates between the sunny and shady sides of the tree before versus after 1 June. The method used in this study to estimate the emission rate examines the amount of active ingredient lost from the device, which may be greater than the amount emitted due to chemical instability or sequestration within the walls of the dispenser. Although there are analytical methods that account for this possibility by measuring the amount of active ingredient recovered from absorbent placed in an enclosed chamber with the dispenser (Mayer and Mitchell 1998), the present estimates serve to illustrate that the rate of emission of the hand-applied devices was more variable over the season compared with the Puffers.

Compared with the hand-held devices, Puffers are more complicated, expensive, and vulnerable to possible mechanical problems. They are also potentially more economical to place in the field because far fewer devices per ha are used. Because the Puffers were active only 12 h each day whereas hand-applied dispensers continuously emitted pheromone, and the number of dispensers per ha and amount of phero-

mone released per dispenser was vastly different between Puffers and hand-applied membrane, it was necessary and appropriate to compare release rates of membranes and Puffers by multiplying per-device hourly release rates by the number of devices per ha. Navel orangeworm mating activity occurs mostly within 2–3 h of the end of scotophase under warmer summer conditions, but begins earlier as temperature decreases (Landolt and Curtis 1982); thus, the hourly rate of pheromone dispensed during scotophase is of primary interest. During the period between dusk and dawn the mean emission rate per hectare per hour was greater in the plots treated with hand-applied devices compared with Puffers for the first 15 d after the first application and the first 27 d after the second, but in other times the amount of pheromone emitted dusk to dawn was greater in Puffers compared with hand-applied devices. A significant reduction in the amount of pheromone dispensed was noted in Puffers in late July and early August of 2004, but this was primarily due to problems with canisters and emission rates returned to normal when the canisters were replaced.

The inflection on the plot of cumulative males captured in untreated control plots indicates that the third flight began in the final week of July. For the next 2 wk, the cumulative number of males captured in plots treated with hand-applied devices was intermediate between that of gridded Puffers and that of peripheral Puffers. For the final three weeks of monitoring with traps baited with virgin females, more males were captured in the plots treated with hand-applied dispensers than in those treated with gridded Puffers. This final 3 wk coincides with the period after the Nonpareil harvest. The decreased suppression of males captured in female-baited traps in this period may indicate that the hand-applied devices were more susceptible to shaking and dust of harvest. (Puffers in Nonpareil trees were moved to an adjacent pollenizer row when their original tree was shaken for harvest.) However, the greater number of males captured in plots treated with hand-applied dispensers between the start of the third flight and the Nonpareil harvest suggests that the hand-applied dispensers did not interrupt sexual communication as effectively as the gridded Puffers, despite the sources being more densely distributed. The ability of gridded Puffers to disrupt chemical communication might have been due to mechanistic differences between the two methods of dispensing pheromone, or it might have been because the hourly release rate at night was lower at important points during the growing season.

The mechanisms involved in successful disruption of mating vary depending on delivery systems and species (Sarfraz et al. 2006) and may include point source competition, camouflage, neurophysiological effects, and shifting rhythms of diurnal response (Cardé and Minks 1995, Sarfaz et al. 2006). The former two mechanisms require an attractive blend, whereas the latter two do not (Cardé and Minks 1995, Sarfaz et al. 2006). For both neurophysiological effects and shifts in diurnal rhythm, it is conceivable that emission of pheromone only during the 12 h including scoto-

phase and not the entire diurnal cycle contributed to greater efficacy in Puffers versus the hand-held device, but further research is needed to clarify this point.

In mating disruption targeted against other species, sometimes less technically efficient formulations are used because they provide acceptable results in a manner that is more cost-efficient or more compatible with grower practices, e.g., use of microencapsulated formulations instead of hand-applied devices (Kovanci et al. 2005). Also, some practitioners use targeted rather than season-long control, by using mating disruption against one flight or generation, and residual insecticides against others (Gut et al. 2004). Treatment with hand-applied dispensers, whereas not suppressing males in female-baited traps as well as gridded Puffers in third flight, resulted in similar damage in almonds compared with gridded Puffers and numerically less damage than peripheral Puffers.

If hand-applied membranes and gridded Puffers result in similar damage, then it is likely that mating disruption treatments with membranes would be more economical and therefore more cost-effective. However, only gridded Puffers were examined further in 2004 because control of navel orangeworm in pollenizer varieties was considered as important as control in Nonpareil. These varieties are exposed to more of the third flight than Nonpareil, and the pheromone trap and mating assay data from the experiment in almonds in 2003 suggested that gridded Puffers disrupted chemical communication in the third flight more effectively than membrane dispensers. In the third mating disruption experiment, in almonds in 2004, gridded Puffers significantly reduced navel orangeworm damage in Nonpareil almonds but not in the pollenizer varieties Carmel or Monterey. Subsequent research suggests that reduction of damage in pollenizer varieties can be obtained using larger treatment blocks or a more complete pheromone blend (our unpublished data). Nonetheless, further research should examine the feasibility of targeted control with mating disruption for reduction of damage in almonds, by using hand-applied devices and/or Puffers starting shortly before the second flight.

In summary, we conclude that 1) mating disruption can significantly reduce navel orangeworm damage in Nonpareil almonds; 2) mating disruption, under the conditions in this study, does not seem promising for reducing navel orangeworm damage to pistachios in mature orchards with abundant navel orangeworm populations, but might help prevent abundant navel orangeworm populations if used from when pistachios first come into bearing; 3) the strategy of concentrating Puffers peripherally around large treatment blocks with no Puffers within the block results in greater damage than hand-applied dispensers or Puffers applied evenly throughout the block; and 4) the hand-applied membranes examined do not disrupt navel orangeworm sexual communication as well as Puffers after the start of the third flight, but may protect Nonpareil almonds as effectively as Puffers.

Acknowledgments

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